



Drug-induced increase of carcinoembryonic antigen expression in cancer cells

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Abstract

Most of gastrointestinal, breast and lung cancer cells express carcinoembryonic antigen (CEA). Therefore, this protein represents a suitable target for innovative diagnostic and immunotherapeutic strategies of various tumours. Presently CEA can be involved in three main approaches concerning cancer detection and therapy, i.e. (a) detection of tumour cells in the peripheral blood, bone marrow or lymph node using reverse transcriptase-polymerase chain reaction (RT-PCR)-based measurement of CEA mRNA; (b) targeting of anticancer agents or radionuclides by tumour-selective anti-CEA monoclonal antibodies (mAbs); (c) use of antitumour vaccines capable of eliciting major histocompatibility complex (MHC)-restricted immune responses against CEA-derived peptides.

Actually, it has been shown that the expression of CEA can be up-regulated by pharmacological agents including, antineoplastic drugs (i.e. 5-fluorouracil), cytokines (i.e. interferons or interleukin-6), differentiating agents (i.e. sodium butyrate) and protein kinase inhibitors (i.e. staurosporine). Therefore, the use of drugs capable of increasing CEA expression, could amplify the sensitivity of diagnostic procedures that rely on CEA determination. Moreover, the same agents could increase the efficacy of vaccines based on immunogenic CEA-derived peptides restricted by the MHC. The purpose of this review is to describe several agents that are able to increase CEA expression and to discuss the rational bases for new strategies in cancer detection and therapy aimed at increasing the expression of tumour-associated antigens. © 2003 Elsevier Ltd. All rights reserved.

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1. Introduction

The carcinoembryonic antigen (CEA) is a glycoprotein containing approximately 50% of carbohydrate with a molecular weight of 180–200 kDa [1–3]. This protein belongs to the large CEA gene family, which includes 29 genes/pseudogenes [4,5]. All genes of the CEA family are located within the long arm of chromosome 19 [6,7]. CEA gene family can be divided into three subgroups, i.e. the CEA subgroup containing seven expressed genes; the pregnancy-specific-glycoprotein (PSG) containing 11 expressed genes and the third untitled subgroup containing six pseudogenes. Components of the CEA subgroup are cell surface bound proteins including CEA and the CEA cross reacting molecules, i.e. non-specific cross-reacting antigen

(NCA), biliary glycoprotein (BGP) and CEA gene family member (CGM-6). As a tumour-associated antigen, CEA is extensively expressed in more than 95% of colorectal, gastric and pancreatic carcinomas as well as in approximately 70% of non-small cell lung cancer, 50% of breast cancer, in mucinous ovarian carcinoma, adenocarcinoma of the penis [8] and endometrial adenocarcinoma. CEA is also present, to a lesser extent, on normal adult human colon and in some foetal tissues [9–12]. In particular, it is expressed on the mature columnar epithelial cells and goblet cells of colon, on pyloric mucous cells of stomach, on squamous epithelial cells of tongue, oesophagus and cervix, in secretory epithelia and duct cells of sweat glands and epithelial cells of the prostate [13].

The molecular basis for this differential expression in different tumours and normal tissues are not completely elucidated. The molecular cloning of cDNA for the CEA and CEA-related molecules was performed by different groups [14–21]. Schrewe et al. [21] reported the isolation

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and characterisation of a cosmid clone that contains the complete coding region of the CEA gene, including its promoter sequence. No classic TATA or CAAT boxes have been found at the expected position upstream of the transcription sites of the CEA or NCA gene [21]. Approximately 400 bp upstream from the translation start within the promoter region confers cell-type-specific expression on a reported gene. Hauck and Stanners [22] also identified several nuclear factor binding sites such as upstream stimulatory factor (USF), Sp1, and Sp1-like factor, interacting with the promoter region of the CEA gene. CEA promoter region isolated from CEA-producing human colorectal carcinoma or normal adjacent mucosa are not qualitatively different [23]. It was further demonstrated that nuclear extracts from CEA-producing colorectal carcinoma cells could equally bind to both CEA promoter region isolated from colorectal carcinoma and from normal adjacent mucosa [23]. These studies suggest that DNA-binding CEA transcription factors are not sufficient to induce high levels of CEA expression in normal tissues. Therefore, some other mechanisms appear to be involved in the notable difference of CEA found in tumours compared with normal colonic mucosa. It has been demonstrated that methylation of CEA gene [24] and/or structural changes of transcription factors that interact with the regulatory elements [22] of the CEA promoter may increase CEA gene expression. Moreover, post-transcriptional modifications could also have a modulatory role.

The analysis of the amino acid sequences of the CEA family revealed that they belong to the immunoglobulin super-family [25]. CEA has been found to play a role as adhesion molecule with an important function in signal transduction in cancer cells. Several CEA subfamily members, including CEA, possess cell adhesion properties [26–30], and their presence in the tumour cell membrane has been considered responsible for increasing the tumour cell dissemination [31–36].

Some authors suggested that CEA molecules expressed on normal cells might play a role in innate immune defence, protecting the colonic mucosa from microbial invasion [37]. Recently, Soeth et al. [38] reported that over expression of CEA protects human colon cancer cells from a variety of apoptotic stimuli, including drug treatment, UV light and confluent growth.

Identification of human tumour-associated antigens (TAAs) and epitopes as target for specific immunotherapy are currently under study. The TAAs that have been characterised in recent years, derive from a broad array of intracellular molecules such as point mutated ras [39], point mutated p53 [40] c-ErbB2, prostate-specific antigen [41], MUC-1 [42], and CEA [43–45]. It is well accepted that MHC-restricted peptides originated from these molecules are capable of being recognised by T cells. For example, CEA protein is processed endogenously and subsequently short peptide segments are presented by HLA alleles on tumour cell surface. Two distinct pathways have been identified for the processing of CEA as an immunogenic

protein. In the first pathway, extra cellular antigen is processed for presentation as 15–25 amino acid length peptides to CD4+ T helper (TH) lymphocytes by MHC class II molecules found on the professional antigen-presenting cells (APCs) including dendritic cells and macrophages. In the second pathway, intracellular antigen produce processed peptides of eight or nine amino acids that are presented to CD8+ cytotoxic T lymphocytes (CTL) by MHC class I molecules (i.e. A2, A3, and A24).

These findings point out that CEA could represent a potential target molecule for specific immunotherapy including recombinant vaccines. A number of preclinical studies demonstrate the possibility to generate *in vivo* a CEA-specific CTL response with anti-tumour activity in mice and in non-human primates. These results have been obtained by using several strategies of immunisation such as recombinant viruses (CEA gene engineered poxoviruses, adenoviruses, etc.), recombinant DNA or anti-idiotypic antibodies [46–49]. A human CTL-mediated response to CEA has been demonstrated by using CEA-derived peptides with HLA-A2.1 binding anchor motifs to stimulate *in vitro* human peripheral blood mononuclear cells (PBMC) derived from normal donors and patients with colorectal carcinoma [45]. As a consequence of such studies, several clinical trials are currently investigating the possible use of CEA as a target antigen, according to different protocols of active specific immunotherapy of patients with colo-rectal carcinoma [50–52]. In spite of encouraging preclinical results, the use of CEA-directed vaccines has been so far unsuccessful in controlling cancer progression in humans. One of the possible explanations is that CEA is heterogeneously expressed in the tumour. Therefore, it cannot be ruled out that a fraction of the entire tumour cell population would not express adequate amount of CEA peptide to be recognised by CEA-specific CTLs.

CEA is widely used as a tumour marker for diagnostic and therapeutic purposes in various neoplasias including gastrointestinal, breast and lung cancer. Since most of CEA positive colorectal tumours shed the antigen in the plasma, measurement of CEA levels is currently used to monitor tumour progression during the post surgery follow-up of patients affected by colorectal cancer. However, it should be noted that not all CEA positive tumours shed the antigen in the serum and no correlation has been found between CEA content in the tumour and CEA levels in the serum. Therefore, in a substantial number of cases this antigen cannot be considered a suitable marker for the detection of sub-clinical residual disease.

Previously, it has been demonstrated that methods based on reverse transcriptase-polymerase chain reaction (RT-PCR) for the detection of CEA positive circulating cancer cells, are sensitive and specific [53–56]. However, limitations of this technique have also been reported in terms of false positive results when RNA was extracted from whole blood [57,58]. Therefore, it has been proposed that epithelial tumour cell enrichment by immunomagnetic beads coated with an antibody raised against a common epithelial

Table 1
Up-regulation of CEA induced by single agents

Drug	Effect in vitro	Reference	Effect in animal model	Reference	Effect in cancer patients	Reference
IFN- α	Increased CEA expression and shedding	[78–83,85]	Increased CEA expression in nude mice bearing human colorectal tumour cell line	[102]	Increased CEA expression; enhanced tumour targeting and radiation delivery to tumour site	[76,77,101]
IFN- β	Increased cell-surface CEA expression and shedding	[79,81,83]	–	–	–	–
IFN- γ	Increased CEA expression and shedding	[80,81,83,84,86–90,93]	Increased CEA expression in nude mice bearing human colorectal tumour cell line	[72,75,91,97]	Increased CEA expression and mRNA of tumour cells isolated from cancer patients	[99,100]
TNF- α	Increased CEA expression and shedding	[112]	Increased CEA expression in nude mice bearing human colorectal tumour cell line	[112]	–	–
IL-6	Increased CEA expression and shedding	[104–106]	–	–	–	–
IL-1 β	Increased CEA expression	[34]	–	–	–	–
TGF β	Increased CEA expression	[108,109]	–	–	–	–
8-Cl-cAMP	Increased CEA expression	[125]	Increased CEA expression in nude mice bearing human colorectal tumour cell line	[95]	–	–
Docetaxel	Increased CEA expression	[116]	–	–	–	–
5-FU	Increased CEA expression and shedding	[84,85,90,91,113]	Increased CEA expression in nude mice bearing human colorectal tumour cell line	[91]	–	–
Staurosporin	Increased CEA expression and shedding	[61,62,128,129]	–	–	–	–
Sodium butyrate	Increased CEA expression	[118,120,122–124]	–	–	–	–
All- <i>trans</i> retinoic acid	Increased CEA shedding	[120]	–	–	–	–
Sodium suramina	Increased CEA shedding	[120]	–	–	–	–
Difluoromethylornithine	Increased CEA shedding	[120]	–	–	–	–
1- <i>O</i> -(2Methoxy) hexadecyl glycerol	Increased CEA shedding	[121]	–	–	–	–
Phorbol ester	Increased CEA expression and shedding	[83]	–	–	–	–

antigen would increase specificity of tumour-associated CEA transcript detection [59–62]. Recently, real-time RT-PCR approach have been introduced as an alternative to conventional RT-PCR for sensitive and quantitative detection of CEA mRNA expressing micrometastasis in the peripheral blood [63], in lymph nodes [64] and in the peritoneal cavity [65] of gastroenteric cancer patients.

CEA has been also used for cancer cell targeting of anti-neoplastic agents or radionuclides bound to tumour-selective anti-CEA monoclonal antibodies (mAbs) [66]. This is an attractive approach either for therapeutic or diagnostic purpose. In fact, anti-CEA mAbs conjugated with ^{125}I have been demonstrated to be safe and effective in tumour localisation in cancer patients [67]. Additional studies have shown that ^{131}I radiolabelled mAbs provide promising therapeutic effects in terms of reduction of tumour mass and metastasis [68–72]. Moreover, CEA expression might be involved in radioimmunoguided surgery for the recognition of tumour cells by anti-CEA radiolabelled mAbs [73,74].

Important factors that appear to limit the use of tumour markers for antibody-based diagnosis and therapy are the low expression of target molecules, the poor penetration of whole antibodies and the formation of human anti-mouse antibodies against to the administered antibody. Therefore, agents capable of increasing CEA expression might represent suitable tools to improve sensitivity and efficacy of diagnostic and therapeutic approaches based on this tumour marker. In particular, pharmacological increment of tumour-associated CEA could be accompanied by higher chances of presenting MHC-restricted CEA-derived peptides by cancer cells. It follows that drug-mediated increase of CEA expression could facilitate the recognition of target cells by cell-mediated immune response induced by anti-CEA peptide vaccines. It was also demonstrated that agents which increase CEA expression can be used to improve either the antitumour effect of radionuclide-conjugated anti-CEA mAbs [72,75,76] or the immunodiagnostic procedures [77] such as radioimmunoscintigraphy or radioimmunoguided surgery based on recognition of this marker. Moreover, drug-induced enhancement of CEA might improve sensitivity of RT-PCR-based

detection of circulating tumour cells from peripheral blood of cancer patients [61,62].

A summary of the agents alone or in combination reviewed in this article and their effects on CEA expression/secretion is shown in Tables 1 and 2, respectively.

2. Agents capable of increasing CEA expression

2.1. Cytokines

2.1.1. Interferons

Attallah et al. [78] observed for the first time that up-regulation of CEA could be obtained in a human colon carcinoma cell line, WiDr, after treatment with a preparation of lymphoblastoid interferon. Further studies have compared the ability of three different human interferons (IFN- α , - β , - γ) to increase the expression of tumour-associated antigen, including CEA, on freshly-isolated human adenocarcinoma cells [79] and on established cancer cell lines [80,81]. The results revealed that IFN- γ was more potent in augmenting CEA levels than either IFN- α or IFN- β . Along with the increased CEA expression, studies have demonstrated also that IFNs are able to induce antigens of the MHC on tumour cells [82–86]. This finding is of potential clinical interest in view of the role that can be played by CEA and the MHC molecules in the recognition of tumour cells by cytotoxic T cells directed against MHC-restricted CEA-derived peptides.

In subsequent reports, the effect of IFN- γ was studied in several human colorectal [87–89] and lung cell lines that vary in both their constitutive level of CEA expression and in their degree of cellular differentiation [86,89]. Conflicting studies have been reported on the correlation between the level of CEA expression and the degree of cellular differentiation [87]. The regulation of CEA by IFN- γ is best demonstrated in the moderately differentiated colorectal tumour HT-29 and WiDr cells. The highly differentiated LS174 cells, which express elevated levels of CEA and the poorly differentiated CEA-negative MIP cells are unresponsive to the ability of IFN- γ to induce changes in CEA contents of either cell membrane or whole cell extract [87]. Furthermore,

Table 2
Up-regulation of CEA induced by agents combination

Drug	Effect in vitro	Reference	Effect in animal models	Reference	Effect in cancer patients	Reference
IFN- γ + TNF- α	Additive effect on CEA induction	[92]	–	–	–	–
IFN- α + IFN- γ + IL-6	Additive effect on CEA induction	[106]	–	–	–	–
IFN- γ + irradiation	Additive effect on CEA induction	[95]	–	–	–	–
IFN- γ + 5-FU	Additive effect on CEA induction	[91]	Additive effect on CEA induction in nude mice bearing human colorectal tumour cell line	[91]	–	–
IFN- γ + hyperthermia	Synergistic effect in the expression of CEA	[96]	–	–	–	–
IFN- γ + IL-6	Synergistic effect in the expression of CEA	[104]	–	–	–	–

the effect of IFN- γ on CEA secretion by human colorectal carcinoma cells was also studied [80]. In this case, the increase of interferon-induced CEA shedding was found to be present in those cancer cells that also show high levels of antigen expression.

The mechanism underlying the effect of interferons on CEA expression is still not entirely elucidated. Data from the literature indicate that signal transduction pathways, through which IFN- γ regulates 2'–5' A synthetase activity, seem to be independent of those involved in CEA regulation. Along with the increased CEA expression induced by IFN- γ in the cytosolic and membrane [84,90,91] cellular compartment, studies have demonstrated elevated levels of antigen-specific mRNA transcripts [91]. It should be noted that although IFN- γ is able to increase also the mRNA level of BGP in a variety of colon cancer cell lines, the mechanisms of induction differ for CEA and BGP [92]. In addition, treatment with Actinomycin D (RNA synthesis inhibitor) or cycloheximide (protein synthesis inhibitor) was followed by the disappearance of the increment of CEA mRNA band in the IFN- γ -treated HT-29 cells [93]. On the contrary, an increased expression of BGP mRNA was induced by cycloheximide treatment. These results suggest that both transcriptional and/or posttranscriptional events could be involved in the up-regulation of BGP by IFN- γ . However, it is unclear whether the increase in CEA mRNA transcripts is due to an increase rate of transcription or, as was the case for HLA class II antigens [91], RNA stabilization, or a combination of both. Although the mechanisms of action remain unclear, IFN increase of CEA levels have been shown not to be due to CEA gene amplification, gene rearrangement or alteration of DNA methylation [24]. Chen et al. [94] demonstrated that the CEA promoter region between –123 and –28 bp upstream from the transcriptional start site, contains binding regions for several important trans-acting factors such as Sp1 and upstream stimulatory factor (USF). However, the footprinting and the gel shift analysis of the CEA promoter showed little differences before and after IFN- γ treatment. Therefore, these results suggest that the majority of IFN- γ effects are at the posttranscriptional level.

Matsumoto et al. [95] demonstrated that CEA expression increased on the surface of a human lung cancer cell line after irradiation either alone or in combination with IFN- γ . The effect IFN- γ and hyperthermia in the expression of CEA was also studied [96]. The combination of IFN- γ with hyperthermia showed a synergistic effect on the expression of the tumour marker.

In vivo studies showed that IFN- γ treatment of mice bearing human colorectal tumour xenografts increased the levels of CEA either in tumour cells or in peripheral blood [72,75,91,97].

An improved efficacy of tumour localisation as well as enhanced antitumour effect of radionuclide-conjugated anti-CEA mAbs have been described as result of IFN- γ -induced expression of CEA. Noteworthy, IFN- γ treatment of fresh human colon cancer cells, obtained from surgical specimens,

resulted in increased CEA levels. On the contrary, no significant change in CEA content was found in adjacent normal tissues after treatment with the cytokine [98]. Clinical studies also showed that in vivo administration of IFN- γ to cancer patients, increases not only CEA expression on human tumour cells [99], but could also enhance tumour shedding and thereby the serum level of the antigen [100]. These results provide additional evidence for the use of cytokines, such as IFN- γ , to improve diagnostic or immunotherapeutic approaches based on CEA molecule.

Since IFN- α has a longer clinical history than IFN- γ , in several studies the effect of systemic administration of IFN- α on tumour-associated antigen levels was investigated in cancer patients. CEA levels were markedly increased in tumour colonic biopsies taken from carcinoma patients treated with well tolerated doses of IFN- α [101]. Subsequent studies demonstrated that administration of the anti-CEA radiolabelled COL-1 mAb in combination with IFN- α , increased localisation intensity and radiation doses at tumour sites in colon cancer patients [76,77]. Other studies showed that IFN- α conjugated to anti-CEA mAbs increases immunoscintigraphy and immunotherapy results in nude mice bearing human tumours [102]. In addition, the ability of IFN- α to enhance the level of CEA in colorectal cancer patients may represent a new approach to increase the sensitivity of the radioimmunoguided surgery technology [103].

2.1.2. Interleukin 6

In order to evaluate and then to optimise CEA increment, additional studies were performed using different cytokines alone or in association. Interleukin 6 (IL-6) as single agent increased CEA as well as HLA class I expression in WiDr and HT-29 cell lines [104–106]. Moreover, adding IFN- γ or IFN- α to IL-6 revealed a synergistic or additive increase in the CEA expression on colon cancer cells [104,106]. Results obtained from Dansky-Ullmann et al. [104] have shown that neutralising antibodies for type 1 interferon inhibited the increase of CEA expression induced by the treatment with IL-6 alone or in combination with IFN- γ . These findings suggest that the ability of IL-6 to induce endogenous IFN- β could be one possible mechanism for IL-6 to up-regulate tumour-associated antigen expression. Increased antigenic expression occurs at concentrations for both cytokines that are 20–100-fold less than that required for each cytokine as single agent. Therefore, the reduction in the amount of each cytokine may have a substantial impact on the toxicity associated with the use of such approach to enhance cell recognition for tumour detection and therapy.

2.1.3. Interleukin 1

Other cytokines were evaluated for their ability to increase CEA expression. Results performed in different laboratories showed that the pro-inflammatory interleukin 1 (IL-1) beta was capable to enhance the level of the tumour marker in human lung cancer cell lines [34] and to increase CEA secretion in colon carcinoma cell lines [107].

2.1.4. Transforming growth factor β

The influence of the transforming growth factor β (TGF β) which is a potent regulator of growth and differentiation, in many cell systems including human colon carcinoma cells, was also examined. TGF β -1 and -2 up-regulate CEA expression and CEA-related gene products in colon cancer lines [108]. Chakrabarty et al. [109] demonstrated that the induction of the matrix adhesion molecules and of CEA by TGF β -1 was associated with activation of protein kinase C (PKC). In fact, the influence of TGF β -1 on CEA expression in cancer cells was abrogated by PKC α antisense RNA [110] or by PKC inhibitors [111]. Therefore, TGF β -1 regulates secretion and expression of CEA and CEA-related glycoprotein, through a signal-transducing pathway associated with PKC isoforms.

2.1.5. Tumour necrosis factor- α

The influence of recombinant human tumour necrosis factor- α (TNF- α) on whole body distribution of labelled anti-CEA mAbs was also studied in human tumour xenografts. This study demonstrated that TNF- α increased radiolocalisation of ^9Y -labelled anti-CEA mAbs in nude mice bearing CEA positive LS174T colorectal carcinoma, while decreasing radioactivity in blood and in other normal organs [112].

2.2. Antineoplastic agents

2.2.1. 5-Fluorouracil

Among the classical antitumour agents, 5-fluorouracil (5-FU) can be considered one of the major components of multi-drug chemotherapy for the treatment of colon and breast carcinomas and many other common human malignancies. 5-FU is an antimetabolite that exerts its cytotoxic effects mainly through inhibition of thymidylate synthase (TS), which prevents formation of thymidylate, required for DNA synthesis and repair. In spite of its frequent application alone or combined with other antineoplastic agents, its efficacy in gastrointestinal cancer appears to be rather limited. However, novel interest for this compound has been elicited by a number of preclinical data indicating that the antimetabolite is able to up-regulate the expression of CEA in tumour cells. Although, a number of agents can enhance the expression of CEA, 5-FU has a major clinical interest, being the drug of choice for the treatment of colorectal cancer. Studies performed in the laboratory of Maas et al. [84] and in our laboratory have shown that 5-FU and 5-fluoro-2'-deoxyuridine (FudR) were able to enhance the expression of CEA in several human cancer cell lines [85,90,113] and in fresh tumour cells obtained from patients with colorectal cancer [98]. Enhancing effect of 5-FU is detectable in different clones derived from colon cancer lines and in breast cancer cells with various basal levels of CEA expression [113]. On the contrary, the antimetabolite was not able to induce expression of the tumour marker in normal or CEA-negative neoplastic cells and in

particular in those of haematopoietic origin [96,113]. The increase of CEA expression, which occurred in membrane and cytoplasmic compartments of colon cancer cells subject to 5-FU treatment, was also accompanied by enhanced antigen shedding [90,113]. In addition, treatment with the antimetabolite is followed by an increase of CEA mRNA, which is responsible, at least in part, of the enhanced CEA levels. In fact, treatment with Actinomycin D, an inhibitor of RNA synthesis, blocked the enhancement of CEA transcript in 5-FU-treated cells. These results are compatible with the hypothesis that 5-FU-mediated increase of CEA mRNA is not due to inhibition of specific transcript degradation [90]. The mechanism of 5-FU-induced increase of tumour-associated CEA expression is based on biochemical induction rather than selection process. This assumption is supported by the finding that clones, derived from HT-29 colon cancer cell line, with low basal level of CEA (i.e. C22.20) are not more susceptible to apoptosis and growth inhibition induced by 5-FU, as compared to HT-29 line or to the clone expressing high basal level of CEA (i.e. C6.6) [113]. The hypothesis that the selection of CEA-positive cells operated by the antimetabolite could be involved in this phenomenon is further weakened if one considers that increase of CEA levels was obtained in C22.20 cell line derived by sub-cloning a clone of HT-29 cells. Therefore, in this experimental setting, the hypothesis of a selection mechanism appears to be remote.

Several studies indicate that 5-FU and IFNs or folic acid (FA) act in synergy to inhibit the growth of tumour cell lines in vitro. Results obtained in two different laboratories have established that in vitro treatment of human colon cancer cell lines (HT-29 and WiDr) with 5-FU in combination with IFN- γ or IFN- α resulted in CEA and HLA class I levels higher than those obtainable with both agents alone [85,91]. In contrast, cell treatment with FA augmented the cytotoxic activity of 5-FU but did not alter CEA modulation induced by the antimetabolite [91]. This data suggest that the FA-sensitive component of the biochemical mechanisms of action of 5-FU is not involved in its effect on CEA expression. Noteworthy, treatment with 5-FU alone or in combination with IFN- γ was found to augment CEA protein in vivo either in colon cancer cells or in peripheral blood of nude mice bearing HT-29 cells [91].

It is of interest to investigate whether treatment with fluoropyrimidines could amplify the efficacy of anti-CEA vaccines in patients bearing CEA-positive tumours. A strong limitation of anticancer vaccines strategy targeting CEA stems from the relative degree of tolerance to these antigens and the low and heterogeneous tumour cell expression of CEA and MHC. Recent results, obtained in our laboratory [114], pointed out that 5-FU treatment increases the killing of colon and breast carcinoma cells in vitro by CTL lines specifically directed against MHC-restricted human CEA peptides. In our experimental model, the enhancing effect of the antitumour agent on immuno-mediated target cell lyses seems to be the result of 5-FU-mediated up-regulation

of target CEA expression. It should be noted that treatment with 5-FU of functional mature CTL after in vitro expansion, did not reduce their cytolytic effect.

2.2.2. Docetaxel

Docetaxel, a semi-synthetic analogue of Taxol, exhibits cytotoxic action by inhibiting mitosis. The drug possesses a unique ability to bind to polymerised tubulin and increase microtubules formation. Docetaxel has significant activity in a broad range of human cancer including drug-resistant ovarian cancer, breast, lung, bladder and head and neck cancer [115]. Docetaxel has been shown to increase the expression of adhesion molecules, including CEA, E-cadherin [116], LFA-3, ICAM-1, CD44s, CD15, CD13 VLA-4/5/6 [117] on human colon carcinoma cell lines. Treatment of the colon carcinoma cells HT-29 with docetaxel increased the adhesion and the immunotoxicity of lymphocytes.

2.3. Differentiating agents

2.3.1. Sodium butyrate

CEA has often been associated with colon carcinomas that have a more differentiated phenotype. Accordingly, a number of differentiation inducing agents [118–122] can increase the expression of the tumour marker. Sodium butyrate (SB) is a short-chain fatty acid that has been found to affect growth and differentiation of a wide variety of tumour cells in vitro, including human colon cancer cell lines. The ability of this agent to induce differentiation is mediated by its inhibition on histone deacetylase that leads to increased histone acetylation and DNA transcription. Several groups have investigated the influence of SB on CEA expression in colorectal carcinoma cell lines. The results showed that SB is good inducer of CEA expression in colon cancer cell lines differing in their degree of differentiation [118,120,122,123]. Saini et al. [124] also showed that the induction of CEA expression by SB is mediated by the increased stability of CEA mRNA. Exposure of HT-29 cells to different inducers including 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) [83] forskolin, and SB resulted in a specific increase of CEA expression [118].

2.3.2. All-trans retinoic acid

One of the most potent agents that induce growth inhibition and differentiation in a variety of human cancer cell lines is an analogue of Vitamin A, the all-*trans* retinoic acid (ATRA). Reynolds et al. determined the effects of ATRA on CEA expression and cell growth in the Moser and HT-29 human colon cancer cells. The results showed that the growth inhibitory effect of the differentiating agent was accompanied by a seven-fold increase of CEA and fibronectin production in both cell lines [120].

2.3.3. 8-Cl cAMP

A series of different agents such as cyclic AMP (cAMP) and related analogues were evaluated for their ability to en-

hance CEA. One analogue, 8-Cl cAMP, was found to suppress the in vitro human tumour cell growth as well as inducing specific changes related to alterations in cellular differentiation. Treatment of the colon cancer cells LS174, HT-29 and WiDr with 8-Cl cAMP increased selectively CEA expression in cell extracts as well as enhanced the level of CEA-related mRNA transcripts. In vivo administration of 8-Cl cAMP to nude mice bearing human tumour xenografts enhanced CEA level and increased tumour localisation [125].

2.3.4. Other differentiation-inducing agents

It has been observed that alkylglycerols, a promising class of natural compounds, of potential value for chemoprevention of malignant diseases, promote a more benign and differentiated phenotype in human colon cancer cells. Treatment of three human colon cancer cell lines with the methoxy substituted alkylglycerol-1-*O*-(2-methoxy) hexadecyl glycerol stimulated CEA production [121]. More recently, a group [119] demonstrated that a human mammary epithelial cell line were sensitive to the association of the differentiation-inducing agent 1,25-dihydroxyvitamin D(3) with TNF- α resulting in an increased expression of a number of tumour markers including CEA. Other differentiation-inducing chemicals such as difluoromethylornithine, sodium suramine also induced increased CEA shedding in colon cancer cells lines, albeit to a lesser degree [120].

2.4. Protein kinase inhibitor

Protein kinases (PK) including protein kinase C (PKC) play an important role in tumour growth, differentiation and spreading. Since CEA appears to be involved in cell adhesion [27,28,30,33,126] and differentiation [127], our group studied the influence of PK inhibitors (PKI) on CEA expression in cancer cells. Colon cancer cells were treated with the tyrosin kinase inhibitor (i.e. herbimycin A) or the protein kinase C inhibitors [i.e. 1-(isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H-7); staurosporine (ST); or bisindolylmaleimide] [128,129]. Among the PKI tested only ST, a non-specific but potent inhibitor of PKC, was capable of increasing substantially the expression of the marker in human colon carcinoma cell lines (WiDr, GEO, HT-29 and its C22.20 and C6.6 subclones expressing low and high basal level of the antigen, respectively) and in breast cancer MCF-7 cells. The increase of CEA expression was demonstrated in terms of (a) plasma membrane and cytoplasm antigen levels; (b) mRNA transcript and (c) protein shedding. It should be noted that ST did not induce the expression of the antigen in CEA negative cells such as leukemic cells or normal PBMC. ST is also endowed with phorbol ester-like properties, being able to induce translocation of PKC isoenzymes from cytoplasm to the membrane and nucleus. To this regard, it should be noted that results obtained by Leon et al. [83] indicated that TPA is able to increase the surface ex-

pression and shedding of CEA by human breast carcinoma cells. Moreover, long-term treatment with TPA results in the complete loss of PKC while the CEA expression was only slightly increased. It must be pointed out that two PKC inhibitors such as bisindolylmaleimide and H-7 were not able to modulate CEA expression. It is conceivable, therefore, that biochemical properties of ST, distinct from those involved in PK inhibition, could be responsible for its effects on the tumour marker. This seems to be further confirmed by the finding that H-7, a potent inhibitor of PKC activity, induced down regulation rather than up regulation of CEA expression and shedding in human adenocarcinoma SW480 cell line [130].

2.5. Glycosylation inhibitor

The glycosylation inhibitor tunicamycin was found to inhibit the cellular glycoprotein synthesis but did not influence CEA levels. However, treatment of human colon cancer cell line LS174T with tunicamycin induced several forms of CEA poorly glycosylated and with molecular weight lower than 200 kDa [131].

3. Conclusion

CEA is present in the digestive organs of the human foetus. However, the majority of gastrointestinal cancer as well as breast cancer and small cell lung carcinoma express CEA. A growing body of literature established that the measure of CEA levels in the plasma could not be considered a suitable marker for the screening of colon cancer [132]. However, there is good evidence that the routine serum CEA determination in post-surgical surveillance of colon cancer reveals early recurrence.

A number of studies showed that cell-associated CEA has been utilised as a tumour marker to trace circulating cancer cells for prediction of recurrence [133] or to detect micrometastasis in lymph nodes and bone marrow [134,135] by means of PCR-based molecular procedures.

CEA has been also used for targeting anticancer agents or radionuclides by tumour-selective anti-CEA mAbs [72,136,137]. The same marker has been utilised for specific immunotherapy including recombinant vaccines able to elicit MHC-restricted immune responses against immunogenic CEA-derived peptides [43,45,47,138,139].

On these bases, the finding that CEA can be up-regulated by pharmacological intervention is of potential clinical interest. Drugs capable of up-regulating CEA might represent suitable tools to improve sensitivity and efficacy of diagnostic and therapeutic approaches, respectively, which utilise CEA as target molecule. It should be pointed out that malignant tumour cell population is highly heterogeneous for CEA expression. However, CEA-inducing agents are able to up-regulate the level of this antigen in various clones expressing different basal levels of the marker [113,127].

Recognition and evaluation of circulating cancer cells in patients with advanced disease has been intensively studied in the last years [53,54,140–144]. In most cases, recognition techniques relied on the detection and quantification of epithelial antigen transcripts such as CEA [53–55,143–151], cytokeratin [54,143,144], mucin [144], uroplakin [152], maspin [153], in circulating tumour cells by either conventional RT-PCR or real-time RT-PCR. Search for CEA mRNA on whole blood for prediction of recurrence or in lymph nodes and bone marrow to detect micrometastasis, is an attractive approach to determine prognosis and therapeutic effectiveness of cancer therapy. However several reports demonstrated that RT-PCR performed with RNA extracted from whole blood might be associated with false positive results [57,58]. Therefore, it has been proposed that tumour cells can be separated from the blood with various methods. In particular, techniques based on the “capture” of epithelial cells from whole blood using immuno-magnetic beads coated with a monoclonal antibody directed against a common epithelial antigen (BerEP4) [59,60], appear to increase specificity of CEA transcript detection based on RT-PCR. Recently, it has been demonstrated [61,62] that *in vitro* treatment with ST of peripheral blood collected from cancer patients increased CEA gene transcription as evidenced by RT-PCR analysis in captured epithelial cells. Moreover, using this approach it was possible to evaluate the presence of micrometastasis in blood specimens collected from patients with metastatic colon cancer before and after chemotherapy [62].

Antitumour vaccines capable of eliciting MHC-restricted T cell-mediated immune responses against the CEA-derived peptides have been described [43–47,49,138,139,154,155]. Our laboratory has spent several years to study the possible influence of drugs on the antigenic makeup of target cells. In this context, it has been demonstrated that (a) triazene compounds are able to induce novel antigens in murine and human cancer cells, through a mutational mechanism, i.e. “chemical xenogenization” [156–159]; (b) antineoplastic agents such as fluoropyrimidines [90,91], ST [128,129] and cytokines induce a marked increase of the expression of CEA in human cancer cells. All these observations, along with other numerous reports of the literature (e.g. CEA up-regulation by interferon-gamma) [72,79,84,91], allow to advance the hypothesis that pharmacological treatment could be responsible for a phenomenon that we have called “drug-induced antigen remodelling” (DIAR, Bonmassar et al., in preparation) of neoplastic cells. This would lead to changes in tumour cell immunogenicity/antigenicity after exposure to antitumour drugs, to cytokines, or to both types of agents (Fig. 1). When DIAR is followed by an increment of immunosensitivity of neoplastic cells to the cytotoxic effects of host’s immune responses, this phenomenon deserves particular attention. In fact, DIAR could provide a new approach to generate clinical protocols for “highly active antitumour immuno-chemotherapy” (HAAIC).

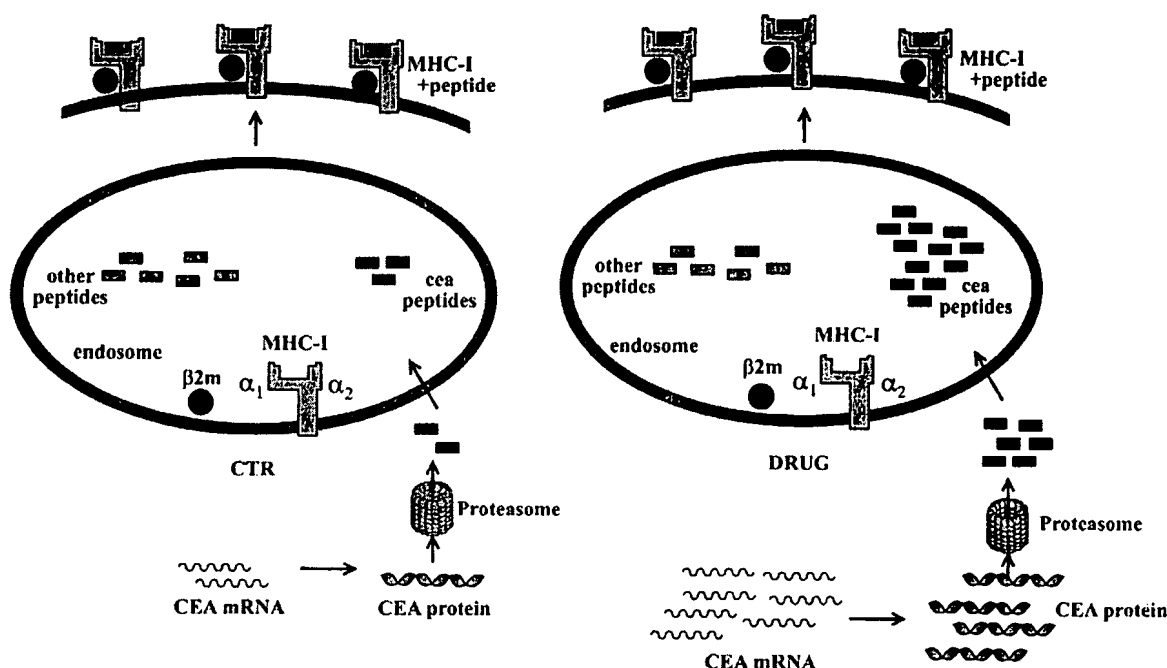


Fig. 1. Schematic diagram of the possible influence of drug-induced CEA up regulation on the antigenic makeup of target cells. Exposure of tumour cells to drugs induces increased endocellular biosynthesis of CEA molecules that would be accompanied by higher chances of presenting MHC-I (Class I major histocompatibility complex)-restricted CEA-derived peptides by cancer cells. CEA peptides are generally presented to T-suppressor/cytotoxic lymphocytes by MHC-I (between α_1 and α_2 domain) associated with β_2 microglobulin (β_2m).

A recent study [114] pointed out that 5-FU treatment enhances the killing of colon and breast carcinoma cells *in vitro* by CTL lines specifically directed against human CEA-derived peptides. In this experimental model, the enhancing effect of the antitumour agent on immuno-mediated target cell lysis seems to be the result of 5-FU-mediated up-regulation of target antigen expression. In fact, it is reasonable to predict that increased endocellular biosynthesis of CEA molecules (as provoked by exposure to the antimetabolite), would be accompanied by higher chances of presenting MHC-restricted CEA-derived peptides by cancer cells, as confirmed by cold competition assay experiments.

All these preclinical studies and the preliminary clinical investigation on the role of pharmacologically-modulated CEA expression, allows to conclude that this tumour marker possesses a particular high potential interest for new approaches in the exogenous control of tumour targets relevant to diagnosis and therapy.

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